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13. ABSTRACT *(Maximum 200 words)*

The objective of this project was to investigate the molecular and cellular processes that regulate the rate of adaptation of photosynthetic light harvesting and nitrogen assimilation capacity of marine diatoms to fluctuating light and nutrient environments. The initial tasks were to develop and verify nucleic acid and antibody probes for assessing gene presence, transcriptional activity and posttranslational modulation of the expression of nitrate reductase (NR), glutamine synthetase (GS) and the fucoxanthin-chlorophyll a-c pigment-protein (FCP) in diatoms. Given the distinct evolutionary history of diatoms we independently cloned segments of these genes using the polymerase chain reaction and developed antisera specific to the diatom forms of these enzymes. Sequence analysis indicates that NR and GS in diatoms exhibit limited homology with the forms occurring in land plants. Concurrent application of the NR nucleic acid probe and antisera indicated that the expression of NR is under strong transcriptional and post translational control when cells are exposed to realistic environmental shifts. NR and FCP were observed to be inversely regulated by shifts in light availability.

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OBJECTIVE: To investigate the molecular and cellular processes that regulate the rate of adaptation of photosynthetic light harvesting and nitrogen assimilation capacity of marine diatoms to fluctuating light and nutrient environments.

ACCOMPLISHMENTS: A major goal of the research was to develop cDNA probes for the genes encoding the rate-limiting enzymes for nitrate and ammonium assimilation, nitrate reductase (NR) and glutamine synthetase (GS), respectively as well as a probe for the diatom light harvesting complex a, fucoxanthin chlorophyll a-c pigment-protein (FCP). Prior attempts to isolate genes encoding NR & GS from diatoms by screening diatom Southern or northern blots with heterologous (ie. higher plant) probes proved unsuccessful; western analysis indicated that heterologous antisera would also not be suitable for screening diatom expression cDNA libraries or for the study of the regulation of these genes by environmental factors. Therefore, we initiated a cloning strategy based on polymerase chain reaction (PCR) amplification of conserved domains of nucleic acid sequences encoding these proteins. Amino acid sequence comparisons of higher plant, fungal and bacteria forms of NR and GS were performed and revealed sets of 6 - 10 conserved amino acid residues which served as suitable primer sites for PCR. Degenerate oligonucleotides corresponding to all possible codon usages for these domains were synthesized and used in PCR amplification of genomic DNA and cDNA from higher plant, green algal, diatom and other chromophytic macroalgal species. Products of the size expected for the regions bounded by the primers where obtained and hybridized strongly to diatom Southern and northern blots. Sequence analysis of these products indicate that although the green algal "control" PCR products generally exhibit greater than 50% identity at the amino acid level to published plant sequences, the diatom and brown algal sequences exhibit less than 40% identity, suggesting that in chromophytes these proteins are significantly divergent from green algae and higher plants. Native gel electrophoresis was used to isolate pure FCP from diatoms and kelp. V8 protease treatment of these proteins followed by amino acid microsequencing

was used to determine internal amino acid domains for designing PCR and hybridization oligonucleotides for FCP. The NR, GS, and FCP clones are currently being used as hybridization probes to examine the dynamics of the transcripts in response to physiologically realistic transients in light and nitrogen availability. The probes are also being used to isolate full length coding sequences for these genes. The NR probe has been used successfully to demonstrate that the accumulation of NR transcripts is light and nitrate dependent (Smith et al. 1992).

We are also interested in the post-transcriptional processes that may regulate the expression of NR, GS and FCP and towards this end have been developing antibody probes against these proteins. An anti-FCP serum, already available, has been used to verify that FCP accumulates under low light conditions, but that this accumulation is not dependent upon the nitrogen source used for growth. A heterologous antisera raised against squash NR was used to demonstrate that in diatoms, enhancement of NR activity in response to light and nitrate enrichment occurs independently of any net synthesis of NR protein, suggesting that light can directly activate existing enzyme (Smith et al. 1992). Due to the low cross-reactivity of this antisera with diatom NR we undertook the purification, characterization and antibody production of diatom NR. Antisera for Skeletonema costatum NR has been obtained and affinity purified; western blot analysis indicates that this antisera is specific to the diatom form of NR. The purified NR will also be used to further examine the nature of the light activation response. A protocol for purification of GS from chromophytic algae based on ion exchange chromatography has been developed and purification for antibody production is underway. In addition, one of our graduate students has developed a protocol for the isolation of high molecular weight and restrictable DNA from Prochloron, the prochlorophyte symbiont of didemnid ascidians (Urbach et al. 1992). This development will enable us to examine the genomic organization of GS and NR in this evolutionary divergent algal group.

SIGNIFICANCE: The development of these molecular and immunological probes specific for marine diatoms will enable us to examine the influence of light and nitrogen availability in modulating gene expression. These studies will determine the relationships between molecular level changes and the physiological expression of these changes as they relate to adaptation to variable light and nitrogen regimes. Comparisons of light and nitrogen dependent gene expression between diatoms and higher plants may also provide us with insights as to the evolution of gene expression control circuits in photosynthetic organisms. Additionally, our development of "universal" PCR primers for NR, GS, and FCP will enable rapid characterization of these genes in a variety of marine algae.

FUTURE WORK: We plan to complete characterizing the NR, GS, and FCP genes in diatoms with the immediate goal of isolating full length genomic sequences. These clones will be used to map and sequence the putative

promoter elements associated with the 5'- regions of these genes and sequence searches will be conducted to determine if these regions are homologous to light responsive promoter elements characterized in higher plants. Application of nucleic acid probes and antibodies in conjunction with measurement of the associated physiological processes in diatom cells subjected to environmentally realistic transitions in their light and nitrogen environment will enable us to define the cellular levels of control on the expression of that physiological process. Measurement of transients in other cellular metabolite pools (amino acids, adenine nucleotides, photosynthetic pigments) will be conducted to determine whether these components play a role in the transduction of environmental signals to gene expression events.

PUBLICATONS:

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